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(71)(72) Applicant and Inventor: NILSSON, Kurt, G., I. [SE/SE]; Andjaktsv. 6, S-222 53 Lund (SE).

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(54) Title: A METHOD FOR SYNTHESIS OF OLIGOSACCHARIDES

(57) Abstract

A method for synthesis of oligosaccharide compounds which either consist of or are fragments or analogs of the carbohydrate part in glycoconjugates is described. The synthesis from donor and acceptor substrates is carried out in that at least one glycosidase and at least one glycosyltransferase are used as catalysts and the oligosaccharide compound is isolated from the reaction mixture.

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A METHOD FOR SYNTHESIS OF OLIGOSACCHARIDES

Description

The present invention relates to a method for enzymatic synthesis of an oligosaccharide compound, which either consists of or is a fragment or an analog of the carbohydrate part in a glycoconjugate. Furthermore, the invention relates to the use of the product prepared by this method.

It has been found that the oligosaccharide part of various glycoconjugates (especially glycolipids and glycoproteins) have a number of important functions in vivo (Biology of Carbohydrates, Vol. 2., Ginsburg et al., Wiley, New York (1984); The Glycoconjugates, Vol. 1-V, Academic Press, New York; S. Hakomori, Ann. Rev. Biochem. Vol.50, pp. 733-64); Feizi, Nature, pp. 314 (1985); S. Hakomori, Chemistry and Physics of Lipids, Vol.42, pp. 209-33). Among other things it was found that

- the carbohydrate structures are important for the stability, activity, localisation, immunogenicity and degradation of glycoproteins;
- carbohydrates are antigenic determinants (for example blood group antigens);
- carbohydrates function as receptors when bound to cell surfaces for pathogens, proteins, hormones, toxins and during cell-cell interactions;
- carbohydrates are important to oncogenesis, since specific oligosaccharides have been found to be cancer-associated antigenic determinants;
- frequently only a smaller sequence (di- or trisaccharide) of the carbohydrate part of the glycoconjugate is required for full biological activity (e.g. receptor activity).

Universities and industry are at present working intensely on developing the use of biologically active oligosaccharides within a number of different fields, such as

- novel diagnostics and blood typing reagents
- highly specific materials for affinity chromatography
- cell specific agglutination reagents
- targetting of drugs
- monoclonal antibodies, specific against e.g. cancer-associated structures
- therapy

Besides the above-mentioned fields, a considerable future market is envisaged for fine chemicals based on biologically active carbohydrates.

The organic chemical techniques used today for synthesis of these carbohydrate structures require an extensive protective group chemistry with many steps of synthesis and expensive catalysts. Low total yields are obtained in these complicated reaction schemes and the technique is not favorable, especially for larger scale work.

Enzymes are nature's own catalysts with many attractive characteristics, such as high stereo-, regio- and substrate selectivity as well as high catalytic efficiency under mild conditions. Today, great hopes are therefore placed in being able to utilise enzymes for large-scale selective synthesis of oligosaccharides with fewer reaction steps and consequently higher total yields than by organic chemical methodology.

Both hydrolases (glycosidases, EC 3.2) and glycosyltransferases (EC 2.4) can be used for synthesis (glycosidases: see Nisizawa et al. in The Carbohydrates, Chemistry and Biochemistry, 2nd Ed., Vol. IIA, pp. 242-290, Academic Press, New York (1970)). With

glycosidases reversed hydrolysis (equilibrium reaction) or transglycosylation (kinetic reaction) are often used to obtain synthesis (see e.g. K.G.I. Nilsson, Carbohydrate Res., Vol. 167, pp. 95-103 (1987)). With transferases a nucleotide sugar (UDP-Gal, CMP-Sia, UDP-GalNAc, GDP-Fuc, etc), which is relatively expensive, is used as donor. Both types of enzymes have advantages:
Glycosidases are abundant and can often be used directly without purification, glycosyltransferases show high regio- and acceptor-selectivity. However, both types of enzymes have disadvantages when used for synthesis. Glycosidases have a low or often wrong regioselectivity which may result in complicated product mixtures and thus purification problems. As a result glycosidases are often not suitable for synthesis of higher oligosaccharides. Glycosyltransferases are often present in small amounts in living cells and are thus often of low availability. Furthermore, as mentioned above, the transferases are cofactor dependent.

One of the objects of the present invention is to use the properties of glycosidases and glycosyltransferases in a favorable way for efficient synthesis of oligosaccharides. This is achieved according to the invention by combining glycosidase-catalysed synthesis of an oligosaccharide compound with glycosyltransferase-catalysed synthesis of the final, higher oligosaccharide. An easily available glycosidase is thus used for synthesis of the shorter oligosaccharide compound and a regiospecific enzyme (i.e. glycosyltransferase) is used when a higher regioselectivity is required, i.e. for synthesis of the final oligosaccharide. This is illustrated in the following scheme (which is not intended to restrict the scope of the invention):

$$\begin{tikzcd}
D_1R_1 + AR_2 \arrow[r, shift left, "\text{glycosidase}"] & D_1AR_2 + R_1 \arrow[l, shift left] \arrow[d, "\begin{array}{l} ND_2 \\ \text{glycosyltransferase} \end{array}"] \\
 & D_2D_1AR_2 + N
\end{tikzcd}$$

(D_1R_1 symbolises donor saccharide (oligosaccharide, glycoside) with α- or ß-bound aglycon (R_1), D_1AR_2 is an O-, C-, N-, S- or F-glycoside of a di- or higher oligosaccharide, ND_2 is a suitable sugar nucleotide (CMP-Neu5Ac, UDP-Gal, UDP-GalNAc, GDP-Fuc, etc) and $D_2D_1AR_2$ is the final oligosaccharide product. The glycosidase reaction can also be an equilibrium reaction. More than one glycosidase and/or transferase can be used for synthesis of higher oligosaccharides.

The substrates are selected with regard to the oligosaccharide which is to be synthesised, and are often commercially available or can be synthesised by organic or enzymatic synthesis and therefore do not restrict the use of the invention.

The enzymes are selected with regard to the final oligosaccharide which is to be synthesised. The enzyme can be used in situ (especially several glycosidases) or after partial or complete purification (especially glycosyltransferases) from their natural environment. The enzyme may be used in soluble form or immobilised to a solid phase by e.g. adsorption, encapsulation, chelation, precipitation or covalent binding. Simultaneous use of glycosidase and glycosyltransferase in soluble form or immobilised to a solid phase (eventually co-immobilised) may be advantageous according to the invention facilitating the

conversion of the intermediate oligosaccharide product (e.g. D_1AR_2 in the scheme above) to the final product oligosaccharide (e.g. $D_2D_1AR_2$ in the scheme above). In this way the method according to the invention gives important advantages compared to previous methods:
purification of intermediary product is not necessary, secondary hydrolysis is minimised (i.e. higher yield) and trisaccharides or higher oligosaccharides can be synthesised in a minimum of "pots" (in some cases one-pot reactions). This is facilitated by the high acceptor specificity of most glycosyltransferases: the transferase in the scheme above does not react with with the wrong isomer of D_1AR_2 or with D_1R_1. Thus, for example, CMP-N-acetylneuraminate-ß-D-galactoside (α2-3)sialyltransferase (EC 2.4.99.4) prefers Galß1-3GalNAcR over Galß1-3GlcNAcR as acceptor and Galß1-4GlcNacR and GalR are poor acceptors (Sadler et al., J. Biol. Chem., Vol. 254, pp. 4434-43).
If glycosides (AR_2) are used as acceptors in the glycosidase-catalysed reaction, a product glycoside is obtained which is easy to purify since no anomerisation of the product glycoside occurs. Furthermore, the same glycosidase may be used for predominant synthesis of several isomers, since the regioselectivity may be changed by the use of different aglycons and by changing the configuration (α- or ß-) of the glycosidic linkage between for example A and R_2 in the scheme above (K.G.I. Nilsson, Carbohydrate Res., Vol. 167, pp. 95-103).
The aglycon R_2 may be an organic compound of varying type (aliphatic, aromatic, heterocyclic, or variations thereof) which is O-,N-, C-, S-glycosidically bonded to A. R_2 may also be glycosidically bound F or an -OH group.
As examples of suitable organic aglycons mention may be made of $CH_3(CH_2)n$-groups (methyl, ethyl, etc.), 2-bromoethyl, allyl, trimethylsilylethyl, 2-(2-carbometoxiethylthio)ethyl, amino acids (seryl, threonyl, asparaginyl, etc) or derivatives thereof, peptides, phenyl, bensyl, nitrophenyl, lipids and analogs thereof.

Examples of α- and ß-glycosidases which may be used according to the invention are D-mannosidases, D-galactosidases, L-fucosidases, N-acetyl-D-galactosaminidases, hexosaminidases and other glycosidases in the EC group 3.2 (Enzyme Nomenclature, Academic Press, 1984).

Examples of sialyl-, galactosyl-, fucosyl-, N-acetyl-glucosaminyl-, N-acetyl-galactosaminyl- and mannosyltransferases which can be used according to the invention are found in the EC group 2.4 (Enzyme Nomenclature, Academic Press, 1984). Recombinant enzymes can be used according to the invention.

The synthesis method according to invention is generally applicable to the synthesis of oligosaccharide sequences included in glycoconjugates (see examples of structures given in references on page 1 one above). Of special interest are the minutest fragments of these structures, which are sufficient to transfer biological activity and the choice of Di and A in the scheme above is determined by this.
Examples of interesting structures are blood group determinants, cancer-associated oligosaccharide structures and structures with biological receptor activity (see references on p. 1).

Some examples of how the invention may be used in actual practice are described in the following Examples, which however are in no way intended to restrict the scope of the invention (abbreviations according to IUPAC-IUB's recommendations, J. Biol. Chem., Vol. 257, pp. 3347-3354 (1982)).

EXAMPLE 1

Synthesis of Neu5Ac(α2-3)Gal(ß1-3)GalNAc(ß)-OEtBr.
GalNAc(ß)-OEtBr was obtained by mixing GalNAc(ß)-OPhNO$_2$-p (1.2 g) in 100 ml sodium phosphate buffer (0.05 M, pH 5.2) with 2-bromoethanol (10 ml) and adding N-acetyl-ß-D--glucosaminidase (EC 3.2.1.30; 70 U).
After 48 h at room temperature 500 mg of GalNAc(ß)-OEtBr was isolated by column chromatography (Kieselgel 60, Merck; methylene chloride-methanol-water). GalNAc(ß)-OEtBr (400 mg) and Gal(ß)-OPhNO$_2$-o (1 g) were suspended in 13 ml 0.03 M sodium phosphate buffer, pH 6.5, and dimethylformamide (4 ml) and 7.2 ml ß-D-galactosidase from bovine testes (2 U; Sigma) were added. After 4 days at 37 °C the product was isolated by column chromatogrphy as above. The fractions containing product was acetylated and further purified by column chromatography. Deacetylation gave 40 mg pure Gal(ß1-3)GalNAc(ß)-OEtBr which was characterised with NMR (^{13}C, ^{1}H).

CMP-Neu5Ac (4 mg, enzymatically prepared) and the above disaccharide (4 mg) were dissolved in 2 ml 0.1 M MES-HCl, pH6.7, CMP-N-acetylneuraminyl-ß-D-galactoside (α2-3)sialyltransferase (EC 2.4.99.4, porcine submaxillary gland, 20 mU, 0.17 ml, Genzyme) was added together with 10 µl Triton X-100 and 2 mg bovine serum albumin. More CMP-Neu5Ac (4 mg) was added after 20 h. After a total reaction time of 72 h at 37 °C 5 mg of Neu5Ac(α2-3)Gal(ß1-3)GalNAc(ß)-OEtBr was isolated by column chromatography (Kieselgel 60, acetonitrile-2-propanol-2.5 M NH$_4$OH and Sephadex G-15). The product was pure according to NMR (^{1}H, ^{13}C) and the structure was confirmed by methylation analysis.

EXAMPLE 2

Synthesis of Neu5Ac(α2-3)Gal(ß1-3)GlcNAc(ß)-OMe.
This substance was prepared analogously. ß-D-galactoside (α2-3) sialyltransferase (0.34 ml, 40 mU) was added to 0.7 ml 0.1 M MES-HCl, pH 6.7, which contained 30 mg Gal(ß1-3)GlcNAc(ß)-OMe (synthesised as described above for Gal(ß1-3)GalNAc(ß)-OEtBr but with GlcNAc(ß)-OMe as acceptor), 10 mg CMP-Neu5Ac, 5 µl Triton X-100 and 1 mg albumin. More CMP-Neu5Ac (10 mg) was added after 30 h. After five days at 37 °C column chromatography as described above gave 10 mg Neu5Ac(α2-3)Gal(ß1-3)GlcNAc(ß)-OMe which was pure according to NMR. The structure was confirmed with NMR and methylation analysis.

The acceptor selectivitity of the glycosyltransferases in several of the examples is such that co-immobilised glycosidase and glycosyltransferase can be used and in some cases one-pot reactions are possible (enzymes, glycosidase substrate and nucleotide sugar which are used are mixed directly or nucleotide sugar and glycosyltransferase are added after the glycosidase). In some cases the glycosidase product is only partially purified (i.e. Sephadex G10 column) before further reaction with the glycosyltransferase.

EXAMPLE 3

Synthesis of Neu5Acα2-3Galß1-4GlcNAcß-OMe.
ß-D-Galactosidase from bovine testes was used as in Example 2 for synthesis of Galß1-4GlcNAc-OMe (the enzyme gives this isomer in addition to the ß1-3 isomer; ß-galactosidas from another source (lactobacillus or sporobolomyces) is used for more specific synthesis of Galß1-4GlcNAc-OMe).
α2-3Sialyltransferase (EC 2.4.99.5) is used for the sialylation.

EXAMPLE 4

Synthesis of Neu5Acα2-6Galß1-4GlcNAcß-OMe.
Synthesis as in example 3 but with α2-6sialyltransferase (EC 2.4.99.1).

EXAMPLE 5

Synthesis of Neu5Acα2-3Galß1-3(Neu5Acα2-6)GalNAcα-OEtBr.
Synthesis as in example 1 using GalNAcα-OEtBr as acceptor and in addition α2-6sialyltransferase (EC 2.4.99.7) as catalyst.

EXAMPLE 6

Synthesis of Fucα1-2Galß1-3GlcNAcß-OMe.
Synthesis as in example 2 but with α1-2fucosyltransferase (EC 2.4.1.69) and GDP-Fuc as donor instead of α2-3sialyltransferase and CMP-Neu5Ac, respectively.

EXAMPLE 7

Synthesis of Fucα1-2Galß1-4GlcNAcß-OMe.
Synthesis as in example 6 but with ß1-4 isomer as acceptor.

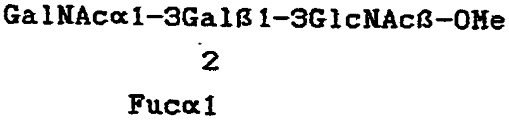
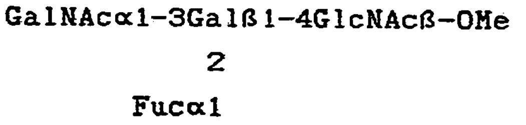
EXAMPLE 8

Synthesis of Galß1-4(Fucα1-3)GlcNacß-OMe.
Synthesis as in example 7 but with α2-3fucosyltransferase (EC 2.4.1.152 or EC 2.4.1.65 with which also Galß1-3(Fucα1-4)GlcNAc can be synthesised as in example 6) as catalyst.

EXAMPLE 9

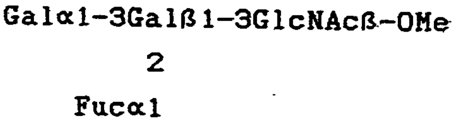
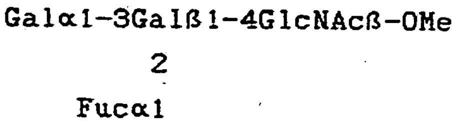
Synthesis of Galα1-3Galß1-4GlcNAcß-OMe.
Synthesis as in example 3 but instead of EC 2.4.99.6 and CMP-Neu5Ac, α1-3galactosyltransferase (EC 2.4.1.151) and UDP-Gal are used.

EXAMPLE 10 and 11


$\text{GalNAc}\alpha 1\text{-}3\underset{\underset{\text{Fuc}\alpha 1}{2}}{\text{Gal}\beta 1}\text{-}3\text{GlcNAc}\beta\text{-OMe}$ (10) and

$\text{GalNAc}\alpha 1\text{-}3\underset{\underset{\text{Fuc}\alpha 1}{2}}{\text{Gal}\beta 1}\text{-}4\text{GlcNAc}\beta\text{-OMe}$ (11).

Synthesis as in example 6 (substance 10; blood group A, type 1) and as in example 7 (substance 11; blood group A, type 2), respectively, and in addition to EC 2.4.1.69, α1-3N-acetylgalactosaminyltransferase (EC 2.4.1.40, from e.g. human milk) and UDP-GalNAc are used.

EXAMPLE 12 and 13


$\text{Gal}\alpha 1\text{-}3\underset{\underset{\text{Fuc}\alpha 1}{2}}{\text{Gal}\beta 1}\text{-}3\text{GlcNAc}\beta\text{-OMe}$ (12) och

$\text{Gal}\alpha 1\text{-}3\underset{\underset{\text{Fuc}\alpha 1}{2}}{\text{Gal}\beta 1}\text{-}4\text{GlcNAc}\beta\text{-OMe}$ (13).

Substance 12 and 13 (blood group B, type 1 and 2, respectively) are synthesised as 10 and 11, respectively, but with EC 2.4.1.37 and UDP-Gal instead of EC 2.4.1.40 and UDP-GalNAc.

In the above examples various methyl and bromoethyl glycosides were prepared. The expert can easily synthesise other interesting glycosides exemplified in the description and choose optimal conditions for the reactions. The sugar nucleotides are obtained with e.g. enzymatic synthesis (nucleotidyltransferase + nucleotide + monosaccharid (or monosaccharide-1-phosphate) for example CTP + Neu5Ac + CMP-Neu5Ac-syntas (EC 2.7.7.43).

Claims

1. A method of production of an oligosaccharide compound which either consists of or is a fragment or an analog of the carbohydrate part in a glycoconjugate from at least one oligosaccharide or monosaccharide glycoside, at least one nucleotide sugar (donor substances) and at least one acceptor substance, characterised in that at least one glycosidase (E.C. 3.2) and at least one glycosyltransferase (E.C. 2.4) are used and that the oligosaccharide compound is separated from the reaction mixture.

2. A method as claimed in claim 1, characterised in that the carbohydrate portion of the donor and acceptor substance includes one or more of the monosaccharides D-galactose, D-mannose, N-acetylneuraminic acid, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and L-fucose, or an analog of these.

3. A method as claimed in claim 1 and 2, characterised in that the acceptor substance is a glycoside in which the aglycon is glycosidically bound flourine or a glycosidically bound aliphatic or aromatic compound.

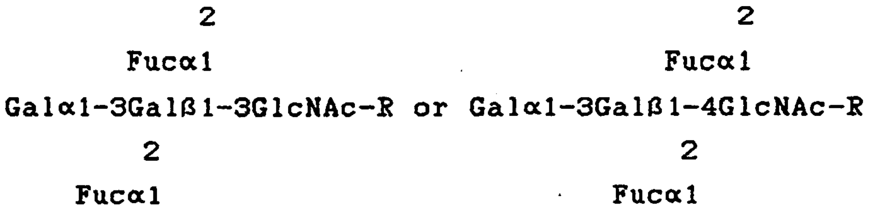
4. A method as claimed in claim 3, characterised in that the aliphatic or aromatic compound is suitable for temporary protection of anomeric carbon, for polymerisation, for construction of affinity labels or of spacer glycosides, for coupling to an amino acid, peptide, lipid(analog), or affinity support, or is an amino acid, peptide, lipid(analog), or is a compound which facilitates use of the oligosaccharide compound as an inhibitor.

5. A method as claimed in one or more of claims 1-4, characterised in that at least one glycosidase and at least one glycosyltransferase in soluble form or immobilised to a solid phase (separately or co-immobilised) are used

simultaneously for the synthesis of the oligosaccharide compound.

6. A method as claimed in one or more of claims 1-5, characterised in that Neu5Acα2-3Galβ1-3GalNAc-R and Neu5Acα2-3Galβ1-3GlcNAc-R are synthesised employing β-D-galactosidase and β-D-galactoside (α2-3)sialyltransferase and R is a glycosidically bound aglycon.

7. A method as claimed in one or more of claims 1-6 characterised in that one of Neu5Acα2-6Galβ1-4GlcNAc-R, Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAc-R, Fucα1-2Galβ1-3GlcNAc-R, Fucα1-2Galβ1-4GlcNAc-R, Galβ1-4(Fucα1-3)GlcNac-R, Galα1-3Galβ1-4GlcNAc-R, GalNAcα1-3Galβ1-3GlcNAc-R, GalNAcα1-3Galβ1-4GlcNAc-R,



$$\text{Gal}\alpha 1\text{-}3\underset{\underset{\text{Fuc}\alpha 1}{2}}{\overset{\overset{2}{\text{Fuc}\alpha 1}}{\text{Gal}\beta 1}}\text{-}3\text{GlcNAc-R} \text{ or } \text{Gal}\alpha 1\text{-}3\underset{\underset{\text{Fuc}\alpha 1}{2}}{\overset{\overset{2}{\text{Fuc}\alpha 1}}{\text{Gal}\beta 1}}\text{-}4\text{GlcNAc-R}$$

is synthesised and that R is a glycosidically bound aglycon.

8. A method as claimed in one or more of the preceding claims characterised in that an oligosaccharide compound having biospecific affinity to another substance is synthesised and isolated.

9. Use of a product obtained in accordance with claim 1, directly or after chemical modification, for affinity chromatography, affinity distribution in two-phase systems, diagnostics, therapy, immunisation, immunological characterisation, changing the properties of enzymes/proteins, measurement of enzyme activity, or modification of recombinant proteins.

AMENDED CLAIMS

[received by the International Bureau on 16 August 1989 (16.08.89)
original claims 1 - 9 replaced by amended claims 1 - 11 (2 pages)]

1. A method of production of an oligosaccharide compound which either consists of or is a fragment or an analog of the carbohydrate part in a glycoconjugate from at least one oligosaccharide or monosaccharide glycoside. at least one nucleotide sugar (donor substances) and at least one acceptor substance. which is a monosaccharide or oligosaccharide. or glycoside or a saccharide analog. characterised in that at least one glycosidase (E.C. 3.2) and at least one glycosyltransferase (E.C. 2.4) are used and that the oligosaccharide compound is separated from the reaction mixture.

2. A method as claimed in claim 1, characterised in that the carbohydrate portion of the donor and acceptor substance includes one or more of the monosaccharides D-galactose. D-mannose. N-acetylneuraminic acid. N-acetyl-D-galactosamine. N-acetyl-D-glucosamine and L-fucose. or an analog of these.

3. A method as claimed in claim 1 and 2. characterised in that the acceptor substance is a glycoside in which the aglycon is glycosidically bound flourine or an O-. N-. C- or S-glycosidically bound aliphatic or aromatic compound.

4. A method as claimed in claim 3. characterised in that the aliphatic or aromatic compound is suitable for temporary protection of anomeric carbon. for polymerisation. for construction of affinity labels or of spacer glycosides. for coupling to amino acid. peptide. lipid(analog). or affinity support, or is an amino acid. peptide. lipid(analog). or is a compound which facilitates use of the oligosaccharide compound as an inhibitor.

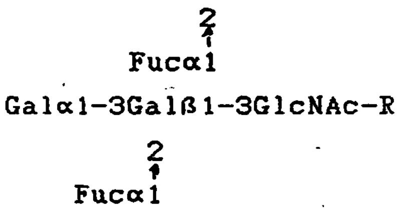
5. A method as claimed in claims 3 or 4. characterised in that the aliphatic or aromatic compound is an allyl-. methyl-. ethyl-. bromoethyl-. epoxi-. trimethylsilylethyl-. phenyl-. bensyl-. or a nitrophenyl group.

6. A method as claimed in one or more of claims 1-5, characterised in that the nucleotide sugar is one or more of CMP-Neu5Ac, GDP-Fuc, UDP-Gal, UDP-GlcNAc, or UDP-GalNAc.

7. A method as claimed in one or more of claims 1-6, characterised in that at least one glycosidase and at least one glycosyltransferase in soluble or immobilised form are used simultaneously for the synthesis of the oligosaccharide compound.

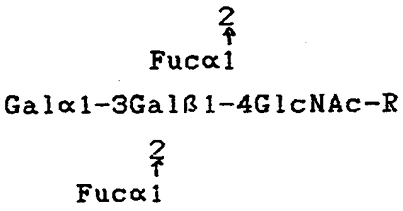
8. A method as claimed in one or more of claims 1-7, characterised in that Neu5Acα2-3Galβ1-3GalNAc-R and Neu5Acα2-3Galβ1-3GlcNAc-R are synthesised employing β-D-galactosidase and β-D-galactoside (α2-3)sialyltransferase and R is a glycosidically bound aglycon.

9. A method as claimed in one or more of claims 1-8, characterised in that one of Neu5Acα2-6Galβ1-4GlcNAc-R, Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAc-R, Fucα1-2Galβ1-3GlcNAc-R, Fucα1-2Galβ1-4GlcNAc-R, Galβ1-4(Fucα1-3)GlcNac-R, Galα1-3Galβ1-4GlcNAc-R, GalNAcα1-3Galβ1-3GlcNAc-R, GalNAcα1-3Galβ1-4GlcNAc-R,



$$\begin{array}{c} 2 \\ \uparrow \\ \mathrm{Fuc}\alpha 1 \\ \mathrm{Gal}\alpha 1\text{-}3\mathrm{Gal}\beta 1\text{-}3\mathrm{GlcNAc}\text{-}R \\ 2 \\ \uparrow \\ \mathrm{Fuc}\alpha 1 \end{array}$$

or



$$\begin{array}{c} 2 \\ \uparrow \\ \mathrm{Fuc}\alpha 1 \\ \mathrm{Gal}\alpha 1\text{-}3\mathrm{Gal}\beta 1\text{-}4\mathrm{GlcNAc}\text{-}R \\ 2 \\ \uparrow \\ \mathrm{Fuc}\alpha 1 \end{array}$$

is synthesised and that R is a glycosidically bound aglycon.

10. A method as claimed in one or more of the preceding claims characterised in that an oligosaccharide compound having biospecific affinity to another substance is synthesised and isolated.

11. Use of a product obtained in accordance with claim 1, directly or after chemical modification, for affinity chromatography, affinity distribution in two-phase systems, diagnostics, therapy, immunisation, immunological characterisation, changing of the properties of enzymes/proteins, measurement of enzyme activity, or modification of recombinant proteins.

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE89/00151

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [8]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

C 12 P 19/04, C 07 H 3/06

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	C 12 P, C 07 H, C 12 M

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Dialog Informational Services, File 5: Biosis 69-89/Jun, accession no. 001116540, Biosis number: 71035393, M.K.A. Markwell et al: "Sendai virus utilizes specific sialyl oligo saccharides as host cell receptor determinants" & Proc Nat (Acad Sci USA, 1980, vol 77 (10), p 5693-5697, abstract	1-9
Y	WO, A1, 87/05936 (H JOHANSSON ET AL) 8 October 1987 See page 2, line 5- page 3, line 30, examples SE, 8601482 SE, 452776 JPT, 63502900	1-9
Y	The Journal of Biological Chemistry, volume 253, no. 16, J.C. Paulson et al: "Sialyl- and fucosyltransferages in the biosynthesis of asparaginyl-linked oligo-saccharides in glycoproteins", pages 5217-5624, see the whole document	1-9

.../...

* Special categories of cited documents: [10]

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 1989-07-06	Date of Mailing of this International Search Report 1989 -07- 1[illegible]
International Searching Authority Swedish Patent Office	Signature of Authorized Officer Niklas Forslund

Form PCT/ISA/210 (second sheet) (January 1985)

International Application No. PCT/SE89/00151

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Glycocojugate research, volume 1, 1979, Proceedings of the fourth international symposium on glycoconjugates, J.C. Paulson et al: "Use of glycosyltransferases and glycosidases in structural analysis of oligosaccharides", pages 247-250	1-9